ADAPTATIONS TO TEMPERATURE IN TWO CLOSELY RELATED STRAINS OF EUGLENA GRACILIS [1]

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A general pattern has emerged from the many studies of physiological adaptations to temperature in protozoans. Within the limits of tolerance, low temperatures result in reduced growth rate and increased cell size, the latter a result of increased amounts of practically all biochemical constituents.

However, significant qualitative differences between species have also been reported. Thus, Johnson (1962) found that respiratory activity in the cryptomonad flagellate *Chilomonas paramecium* increased exponentially with temperature, while Buetow (1963) showed that respiration in a colorless mutant of *Euglena gracilis* var. *bacillaris* increased in a linear manner with temperature. The latter finding is of particular interest as an example of a continuously decreasing Q_{10} with increasing temperatures.

Qualitative differences of this sort must be an expression of genetic diversity among flagellates in a most fundamental aspect of protozoan physiology. Because of this, it seemed desirable to repeat Buetow's work with a wild-type *Euglena*. This report describes some physiological properties of two strains of *Euglena gracilis*, separated only by minor taxonomic differences, as a function of incubation temperature. These studies show marked quantitative but no qualitative differences in temperature adaptation between the two strains. Buetow's report of a linear increase in respiratory activity with elevated temperatures is confirmed when exogenous acetate is available, but a markedly different pattern was observed in endogenous respiration. Other parameters—mass, protein, and RNA—respond in the expected manner.

Methods

Original stocks of the cells used, *Euglena gracilis* strain Z and *Euglena gracilis* var. *bacillaris*, were obtained from Dr. J. A. Gross and have been maintained through serial culture by the author. Growth rates of these stocks, measured under the same culture conditions, have remained constant over a period of years. For these studies, however, a single colony of each strain was picked off agar, inoculated into liquid media, and the resulting populations used. These two clonal populations, derived from single cells, did not differ in growth rate from the parent populations.

The salt medium of Cramer and Myers (1952), with sodium acetate (25 m*M*) as sole carbon and energy source, was used exclusively. Axenic cultures were

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grown in the dark in cotton-stoppered erlenmeyer flasks, maintained in water-jacketed incubators working against an ambient temperature of 10° C. Washed air was flushed continuously through the incubators.

Strain Z was examined at temperatures of 15°, 20°, 25°, 29°, and 34° C. *E. gracilis* var. *bacillaris* grew well enough at 15° C., but formed many clumps of cells (palmella) which made quantitative studies impossible at this temperature; this variety was studied at 17.5°, 20°, 25°, 29°, and 34° C. Temperatures above 32° are supra-optimal for *E. gracilis* and lead to irreversible bleaching. At each temperature, the cells were allowed to adapt through at least 15 generations before measurements were made. Population increase was followed by periodic cell counts with the Coulter cell counter. The cells were always harvested at a population density of 10^5 cells per ml., well below levels of the stationary phase.

At most of these temperatures, cells were analyzed in terms of growth rate, mass, protein and RNA content, and respiratory rates, the latter both at the temperature of incubation and also at a test temperature of 25° C. For the respiration measurements, cells were harvested by gentle centrifugation (Buetow, 1961), washed three times with fresh culture medium (without acetate) and made up to volume in this wash medium. Oxygen consumption of the cell suspension was followed with the Beckman oxygen electrode, using a water-jacketed reaction vessel with constant stirring of the cells by a magnetic bar; with the electrode in position, this vessel was air-tight and contained no gas phase. Depletion of oxygen was recorded graphically, and absolute amounts of oxygen consumed calibrated against air- and nitrogen-flushed water. The temperature of the vessel was either 25° C. or the incubation temperature, held constant by circulating water from a refrigerated bath through the outer jacket of the reaction vessel. Endogenous respiration was followed for 20–30 minutes, after which acetate to 25 m*M* was added and respiration again followed for 20–30 minutes. Over this period of time, no extensive precautions against bacterial contamination were necessary. At the end of such a run, aliquots of the cell suspension were taken for cell counts, so that the respiratory rates could be referred to the average cell. Procedures for other measurements have been described previously (Cook, 1961).

Results and Discussion

Growth rates

Generation times of the two cell types at the several temperatures are shown in Figure 1. At most temperatures, the Z strain of *Euglena* exhibits a faster multiplication rate than var. *bacillaris*. The degree of difference is not constant, however; at 29° C. (the optimum for both cells), the generation time of var. *bacillaris* is the greater by a factor of about 1.48; at 20° C. only 1.1. *E. gracilis* var. *bacillaris* does not respond as readily as strain Z to changes in temperature, at least in terms of multiplication rate. Figure 1 also shows that supra-optimal temperatures (34°) retard cell division more severely in the Z strain.

Cell mass

Figure 2 shows changes in total dry mass in cells adapted to the various temperatures. Between 20° and 29° C. var. *bacillaris* has the larger mass, but

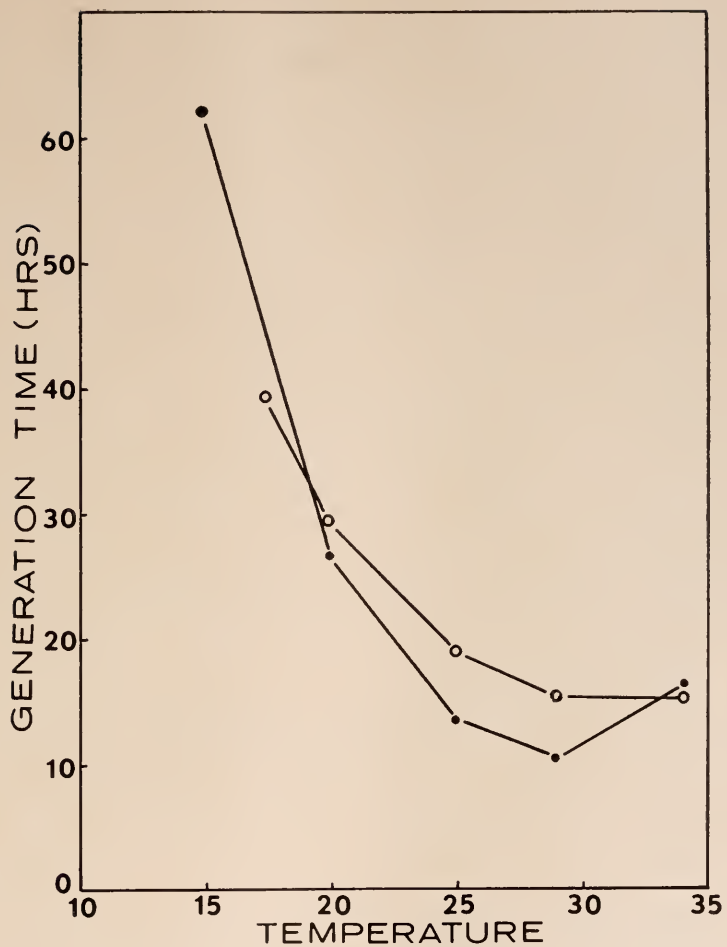

FIGURE 1. Generation times during logarithmic growth as a function of incubation temperature in *E. gracilis* strain Z (●) and var. *bacillaris* (○).

this condition is reversed below 20° C. Strain Z shows a minimum mass at 25°, but the change in var. *bacillaris* is essentially linear over the temperature range examined.

Protein and RNA

The protein content of these cells does not form a constant fraction of cellular mass. As the incubation temperature is changed, the protein fraction ranges between 20% to 30% of the total mass. However, the protein content in both cells changes in the same direction as total mass, being much increased at lower temperatures. Since the polysaccharide paramylum will make up most of the remaining mass, it is assumed that levels of paramylum must also change with temperature, and in the same direction as protein.

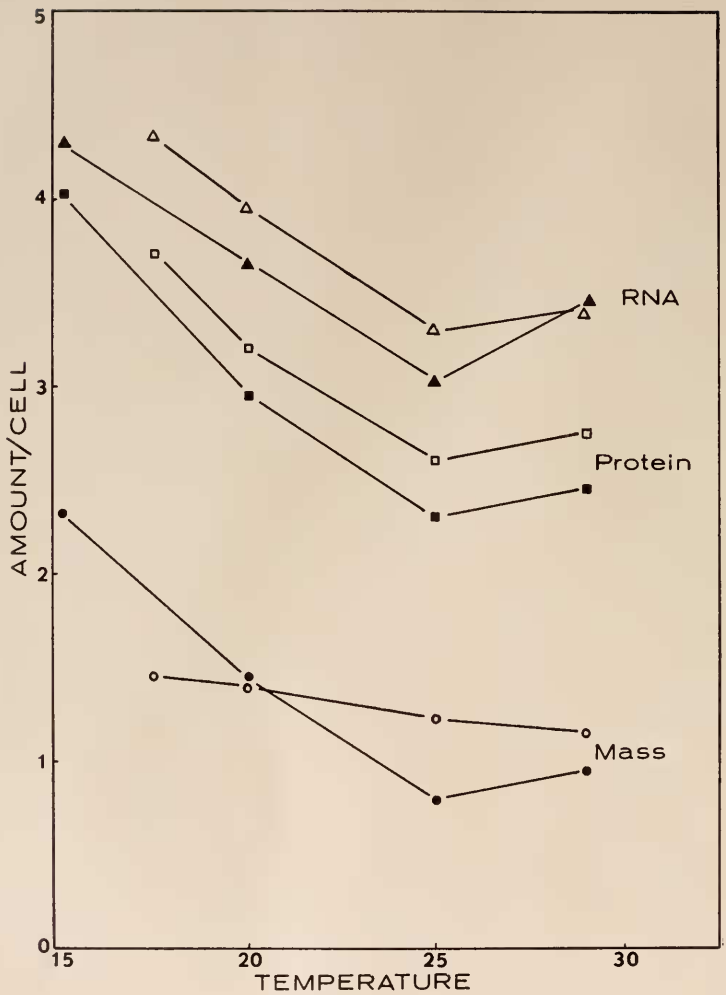

FIGURE 2. Average cell mass (circles), protein (squares), and RNA (triangles) in *E. gracilis* strain Z (filled figures) and var. *bacillaris* (open figures) during log growth at various temperatures. Ordinate values are in $\mu\mu$grams and for the average cell should be multiplied by 10 (RNA), 100 (protein) and 1000 (total dry mass).

RNA levels roughly parallel protein content, being minimum in both strains at about 25° C. In *E. gracilis* var. *bacillaris,* the RNA content is equal to or slightly greater than that of the Z strain (Fig. 2).

Respiration

Figures 3 and 4 summarize respiratory characteristics. At the temperature of incubation, the respiratory rate in the presence of acetate is always greater in the Z strain, by an amount which is nearly constant at all temperatures (Fig. 3).

It may be noted from Figure 3 that these rates increase with temperature in a linear, rather than exponential, manner. In the absence of adaptive changes in respiratory machinery, an exponential pattern would be expected. Since this was not observed, it follows that the respiratory capacity of cells adapted to low temperature must be greater than those adapted to higher temperatures (Precht's type 1). That this is the case was demonstrated by the respiratory rates at a test temperature of 25° C. (Fig. 4). Cells incubated at the lower temperatures consume as much as 50% more oxygen at 25° than do those cultured at higher temperatures, in the presence of exogenous acetate.

Endogenous rates of respiration are essentially the same in both strains of *Euglena*. It is of interest to note that these rates do not change appreciably at temperatures below 25° C. when tested at the temperature of growth (Fig. 3). Endogenous respiration thus shows complete adaption to incubation temperature (Precht's type 2). There is a slight increase in the endogenous rate above 25°. Complete adaptation of this sort would also give rise to increased respiratory capacity at the lower temperatures, to an extent greater even than that found in the incomplete adaptation in the presence of exogenous substrate. That this is the case is seen in Figure 4. The endogenous consumption of oxygen of cells grown at 15–17.5° is about twice as great at 25° as that of cells grown at 25° C. It is concluded that the endogenous response of *Euglena* to temperature differs qualitatively from the response in the presence of exogenous acetate.

Rates of cellular processes

Rate constants for population expansion can be obtained from the generation times by use of the familiar growth equation, k = ln 2/generation time. Synthetic

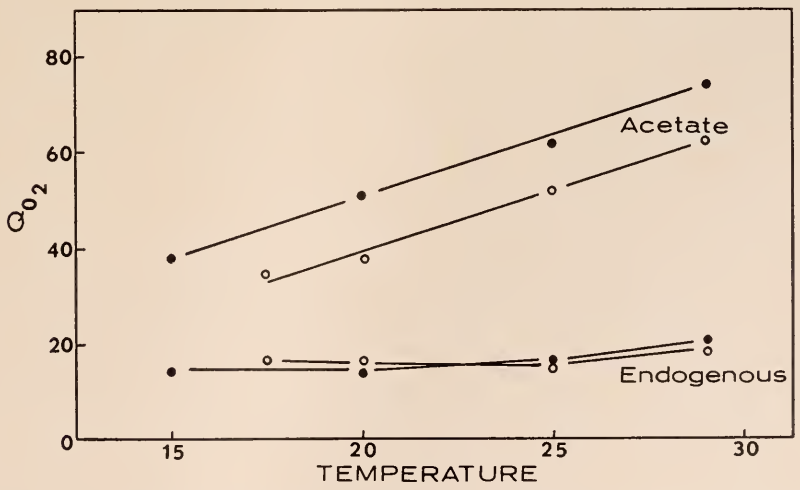

FIGURE 3. Oxygen consumption by *E. gracilis* strain Z (filled circles) and var. *bacillaris* (open circles) during log growth at the temperature of incubation. The lower curves show endogenous consumption, and the upper curves show consumption in the presence of exogenous substrate (acetate). Q_{O_2} = μl. O_2/hr./10^6 cells.

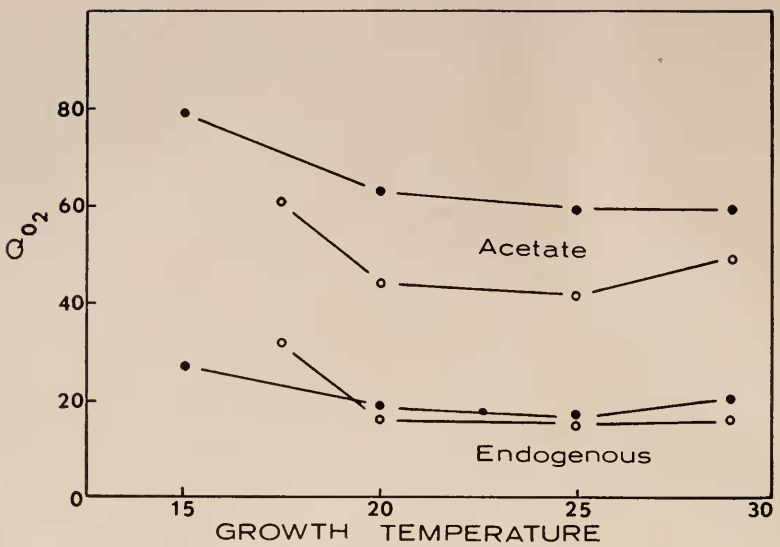

FIGURE 4. Rate of oxygen consumption at 25° C. by *E. gracilis* strain Z (solid circles) and var. *bacillaris* (open circles) after adaptation to growth at the temperature shown on the abscissa. Lower curves, endogenous rate; upper curves, rate in the presence of exogenous acetate. Q_{O_2} as in Figure 3.

rates can be estimated from

$$k = \frac{\overline{M}}{1.44\ GT,} \qquad (1)$$

where k is the rate value (in amount synthesized per average cell per hour), GT is the generation time, and $\overline{M}$ is the amount held by the average cell in the constituent of interest (Cook and James, 1964). Synthetic rates for total mass, protein, and RNA for both strains of *Euglena* at the several different temperatures were calculated from this equation. Figure 5 is a logarithmic plot of these rates. The rate of mass accumulation by the average cell increases exponentially with temperature up to 29°; this rate increases rather more rapidly in the Z strain above 20° C. A more striking difference is seen in the rates of protein and RNA synthesis. While these rates increase exponentially with temperature in both cell types, the rate of increase in the Z strain is considerably greater than in var. *bacillaris*. In both strains, the rate of protein synthesis parallels the rate of RNA synthesis. At about 20°, the two cell types have equal rates of RNA and protein synthesis.

Q_{10} *values*

The Q_{10} values for these various processes can be read from the data shown in Figure 5. They are listed in Table I. The Q_{10} is approximately 2 between 15–17.5° and 29° C. for the rates of mass, protein, and RNA accumulation in var.

bacillaris and for mass accumulation in strain Z; the rates of protein and RNA synthesis in strain Z have Q_{10} values of 2.9 and 3.9, respectively.

The Q_{10} for division rate decreases from 3.3 to 1.3 over the range 17.5°–29° C. in var. *bacillaris* and drops to about 1 between 29° and 34° C. Strain Z is more sensitive to temperatures in terms of division rate, showing a progressive decrease in Q_{10} from 5.6 to 1.7 as the temperature is elevated from 15° to 29° C. (Table I). Above 29° C. the Q_{10} is less than unity in strain Z.

In the presence of exogenous acetate, the respiratory Q_{10} for strain Z is about

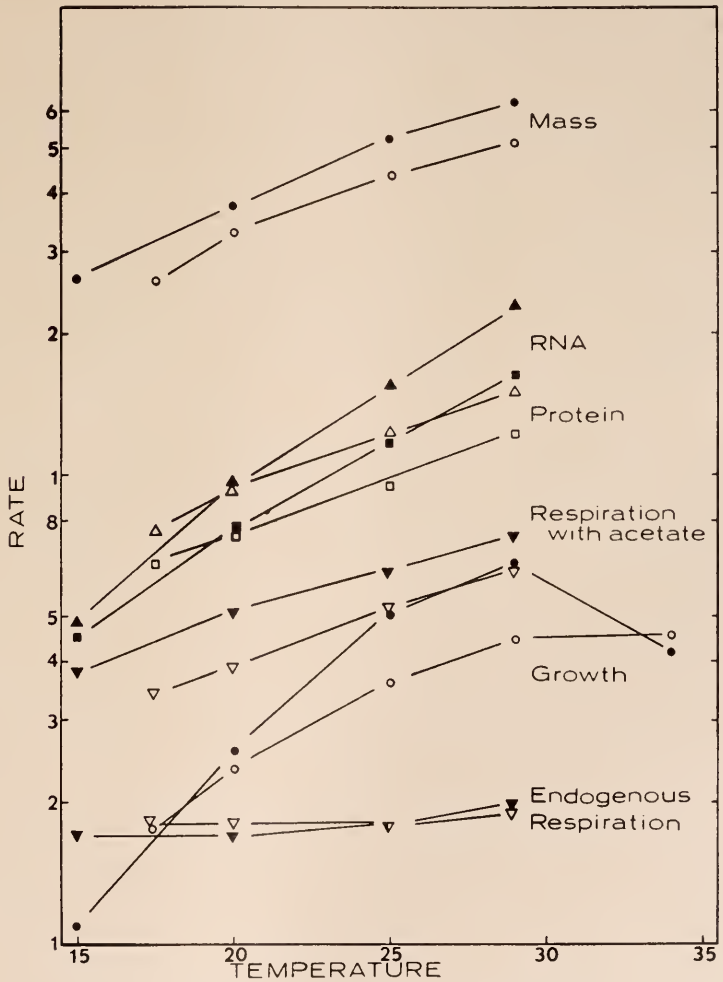

FIGURE 5. Log plot of metabolic and synthetic rates in *E. gracilis* var. *bacillaris* (open figures) and strain Z (filled figures). Rates calculated as described in text. Ordinate values for respiration (μl. O_2/hr./10^6 cells, inverted triangles) are to be multiplied by 10; for growth rate (hr.$^{-1}$, circles) by 10^{-2}; for RNA (triangles), protein (squares), and mass (circles), all in $\mu\mu$gm./cell/hr., by 1, 10, and 10, respectively.

TABLE I

Q_{10} values for various rates in two strains of E. gracilis

Temperature range	15(17.5)–20		20–25		25–29		29–34	
Cell strain	Z	b	Z	b	Z	b	Z	b
Growth rate	5.60	3.26	3.93	2.33	1.71	1.32	.41	1.05
Endogenous respiration	1.00	1.00	1.15	1.00	1.28	1.16		
Respiration (acetate)	1.84	1.73	1.49	1.73	1.49	1.61		
Mass increase	2.00	2.50	2.00	2.12	1.53	1.51		
Protein synthesis	2.90	1.74	2.35	1.74	2.35	1.74		
RNA synthesis	3.85	2.25	2.64	1.70	2.64	1.70		

1.5 between 20° and 29° C., and 1.8 between 15° and 20° C. Under comparable conditions, *E. gracilis* var. *bacillaris* shows an unchanging Q_{10} of 1.7 between 17.5° and 29° C.

It will be noted that respiratory rates at the temperature of incubation are shown on a linear scale in Figure 3, and on a semi-log plot in Figure 5. For *E. gracilis* var. *bacillaris,* a straight line satisfied the experimental points in both cases, which is merely to say that in a biological system it is difficult to demonstrate whether a function is linear or exponential when the range of values differs by no more than a factor of two. In the present case, the Q_{O_2} at 29° is 63 and at 17.5° it is 34.

The Q_{10} for endogenous respiration is about 1 in both strains at temperatures below 25°, and only slightly greater between 25°–29° (Table I). It was expected that respiratory activity in the presence of exogenous substrate (acetate) would be greater than the endogenous level, since acetate should stimulate activity of the Krebs' cycle enzymes. It was quite unexpected to find that the degree of stimulation was not constant as a function of temperature. The rate of oxygen consumption will be in part a function of the level of respiratory enzymes, excluding oxidative reactions which are not directly involved in respiration. That the activity of these enzymes is adaptively increased at lower temperatures is implied by the data for endogenous respiration shown in Figure 3. If the stimulatory role of exogenous acetate were strictly confined to respiratory activities, the Q_{O_2} with added acetate should be reflected in a Q_{10} nearly equal to that of the endogenous. That this is not the case is strong presumptive evidence for the view that oxygen is utilized in non-respiratory functions of *Euglena* cultured on acetate. Supporting evidence of a comparative nature is also suggested by the data shown in Figure 3: both strains of *Euglena* have essentially the same endogenous rate of oxygen consumption, but are stimulated to quite different levels of oxygen consumption by exogenous acetate.

Danforth and Wilson (1961) have shown that endogenous respiration of *Euglena* continues in the presence of exogenous substrate. The Q_{O_2} of *Euglena* adapted to growth and respiration on exogenous glucose is no greater than the endogenous Q_{O_2}, the latter having a level equal to the endogenous rate of acetate-grown cells (Cook and Heinrich, 1965). The optimal growth rate and the cellular mass and protein content are the same when cultured on either substrate. These

data taken together suggest that acetate-grown *Euglena* may consume oxygen *via* two principal routes: one as terminal electron acceptor in respiration, and the other in some non-respiratory and non-energy-yielding reaction(s) associated with growth on acetate.

Growth rate and synthetic rate

Schaechter *et al.* (1958) showed in the bacterium *Salmonella typhimurium* that mass and RNA levels were a positive exponential function of the growth rate but quite independent of temperature between 25° and 37° C., when the growth rate was varied by culture on different carbon sources. Since temperature affects growth rate as well as other physiological rates in *Euglena* with only acetate as carbon and energy source, it was of interest to know whether the observed effects were due primarily to temperature or indirectly to altered growth rates. The present data have been examined to determine whether the biochemical and physiological profiles of *Euglena* conform to some general pattern of the sort described by Schaechter *et al.* No such pattern was found. Figure 6 shows mass and RNA as a function

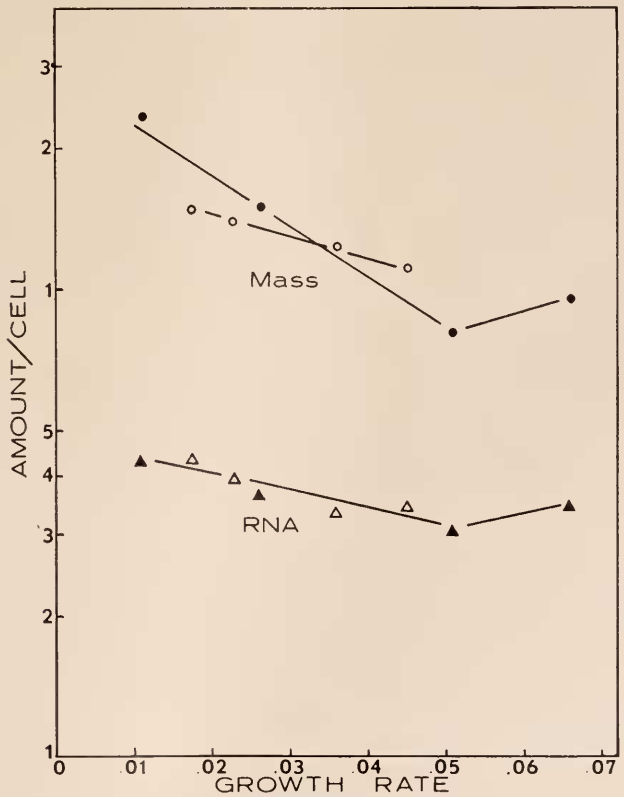

FIGURE 6. Log plot of mass and RNA content of *E. gracilis* strain Z (filled figures) and var. *bacillaris* (open figures) as a function of growth rate. Ordinate values for RNA are to be multiplied by 10 and those for mass by 1000 to give the amount in $\mu\mu$gm.

of growth rate, plotted for both *E. gracilis* var. *bacillaris* and strain Z. The data for protein are quite comparable (*cf.* Fig. 2) but are not shown in Figure 6 for purposes of clarity.

Mass and RNA levels in *Euglena* are a *negative* exponential function of the growth rate when the latter is .05/hr. or less, and a positive function at higher growth rates. In this respect it is noted that the growth rates of *S. typhimurium* as studied by Schaechter *et al.* (1958) were always considerably greater than those reported here for *Euglena.*

It is of interest to note that a single line satisfies the RNA content as a function of growth rate for both varieties of *E. gracilis.* In spite of the fact that strain Z

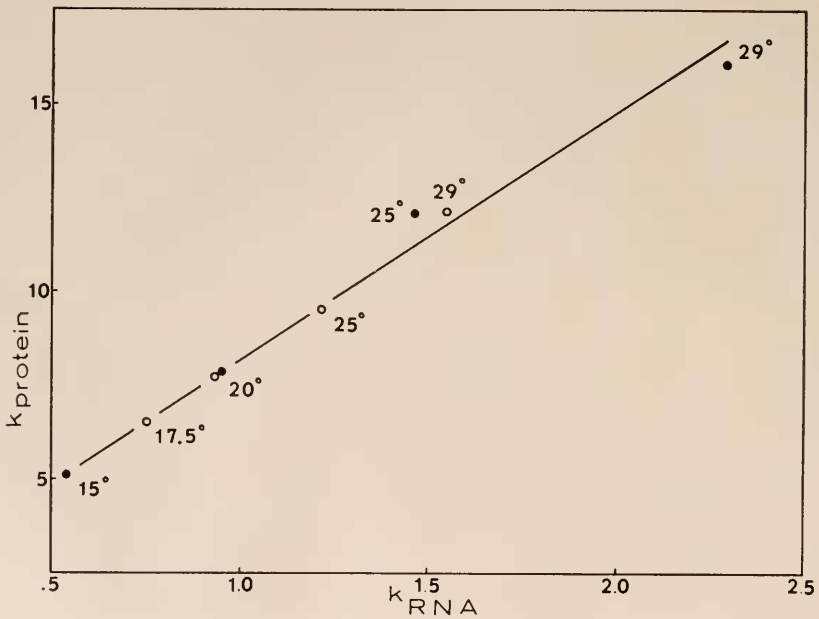

FIGURE 7. Relationship between the rates of RNA and protein synthesis in *E. gracilis* strain Z (filled circles) and var. *bacillaris* (open circles) at various temperatures as indicated. Rates in $\mu\mu$gm./cell/hr.

and var. *bacillaris* can hold widely different values for RNA content and growth rate at a given temperature (*cf.* Figs. 1 and 2), the conformity shown in Figure 6 suggests that genetic differences in the two strains are not yet great enough to be expressed as some divergence in the fundamental relationships between division and synthesis. This view is strengthened by the comparison shown in Figure 7, which is a plot of the rate of RNA synthesis against the rate of protein synthesis for the two strains, when these rates are varied by temperature. Again, a single line satisfies both sets of data. The rate of information translation is the same in both cell lines, at least at a very gross level. By exclusion, it is inferred that the two strains may differ principally in the rate of information transcription. While DNA levels were not followed in the present study, it can safely be assumed that

the Q_{10} for the over-all rate of the DNA synthesis will be exactly equal to that of the growth rate. While DNA replication in the single cell is usually a discontinuous process, variable lengths of the S period could very well determine physiological and biochemical differences of the sort described in this paper, especially since DNA is presumed to be non-functional in RNA synthesis during its own replication (Prescott and Kimball, 1961). The possibility of ploidy is not excluded. It is suggested that the more sluggish behavior of *E. gracilis* var. *bacillaris* when compared to strain Z is the result in part of a slower rate of information transcription.

Summary

1. Certain biochemical and physiological parameters in two closely related strains of *Euglena gracilis* (strain Z and var. *bacillaris*) have been examined after adaptation to various incubation temperatures.

2. The growth rate for the two strains differed at all temperatures, but was greatest in both at 29°.

3. Temperatures below optimal resulted in increased mass, protein, and RNA levels. In general *E. gracilis* var. *bacillaris* was larger in all these fractions at any given temperature.

4. Endogenous respiration proceeded at rates which were essentially unchanging over the temperature range 15°–29° C. Both strains exhibited the same rate.

5. Oxygen consumption in the presence of exogenous substrate (sodium acetate) increased in a linear fashion with the temperature of incubation, rates in strain Z being considerably greater than in var. *bacillaris*.

6. Mass, RNA, and protein content were found to be an exponential function of the growth rate, with a change in the sign of the slope at a growth rate of .05/hr. The rate of protein synthesis was a linear function of the rate of RNA synthesis in both strains.

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